Norwegian Institute for Water Research

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Quality Assurance Project Plan (QAPP)

- for the full scale testing of the PureBallast treatment system of Alfa Laval/Wallenius Water AB

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Abbreviations and acronyms

APHA – American Public Health Association
A. salina – Artemia salina
Wallenius AOT Module – Wallenius Advanced Oxidation Technology Module
Bo, Bi – respectively outer and inner width of each rack with the Pure ballast test equipment
B. submarina – Brachiomonas submarina
CFDA-AM - 5-carboxyfluorescein diacetate acetoxymethyl ester
COD – chemical oxygen demand
CT1 – temporary holding tank on control cycle
CT2 – storage tank on control cycle
DNV – Det Norske Veritas
DO – dissolved oxygen
DOC – dissolved organic carbon
DQIs – data quality indicators
EC_{50}, EC_{10} - the concentrations causing 50 and 10 % effect, respectively, on the test organism
E. coli – Escherichia coli
FNU – Formazine Nephelometric Units
GF/F – glass fiber filter grade F
GLP – Good laboratory Practice
Ho, Hi – respectively outer and inner height of each rack with the Pure ballast test equipment
IMO – International Maritime Organization
ISO – International Organisation for Standardization
LC50 – the concentration causing 50 % mortality of the test organism
Lo, Li – respectively outer and inner length of each rack with the Pure ballast test equipment
n – number of measurements; in calculating the standard deviation
NDIR - Nondispersive Infrared
NIVA – Norwegian Institute for Water Research
NS-EN ISO – Norwegian, European and International Standard
OECD – Organisation for economic Co-operation and Development
PAR – photosynthetic active radiation
POC – particulate organic carbon
PSU – Practical Salinity Unit (= ‰)
QAPP – quality assurance project plan
QA/QC – quality assurance/quality control
QMP – quality management plan
S1-S5 – sampling points 1-5
Std – standard deviation
TCBS - MacConkey and thiosulphate citrate bile salt agar
TSS – total suspended solids
TT1 – temporary holding tank on test cycle
TT2 – storage tank on test cycle
WST – tank with prepared test water
X_i - individual analytical result; in calculating the standard deviation
X – the arithmetic mean of individual analytical results; in calculating the standard deviation

1. Project description, objectives and organisation

1.1 Address of QAPP

This QAPP describes the implementation of quality assurance and quality control activities during the evaluation of the Alfa Laval Pureballast water management system according to the requirements for biological and toxicity testing stated in the IMO Guidelines for Approval of Shipboard Ballast Water Treatment Systems (MEPC 53/24/Add.1, Annex 3, 2005). The requirements involve all parts and processes of the test as listed in:

Annex - Guidelines for approval of ballast water management systems (G8)

- Part 2 – Test and performance specifications for approval of ballast water management systems
- 2.1 Quality assurance and quality control procedures (page 15)
- 2.3 Land-based Testing (pages 17-22)
- 2.4 Reporting of Test Results (page 22)
- Part 4 – Sample analysis methods for the determination of biological constituents in ballast water
- Section 4.1 – 4.8 (page 24-26)

Annex 4 MEPC 53/25/Add.1 - Procedure for approval of ballast water management systems that make use of active substance (G9)

- Section 1-5 (pages 1-7)

The QAPP is a mean to reveal any problems before start-up and during execution of the project at as early stage as possible to minimize any potential procedural, technical and scientific inadequacies and time- and economic losses. This QAPP will be used for biological testing in accordance with the IMO guidelines as well as for the final and basic approval testing of active substances.

1.2 Processes to be evaluated

The QAPP will cover all parts of the project, both preparations, execution of tests and reporting of results;

1.2.1 Preparations

- Construction and preparation of the Test Site according to the IMO guidelines (MEPC 53/24/Add.1, 2005) and advices and instructions from the US Coast Guard/ETV (Battelle Duxbury, 2004).
- Installation and preparation of the PureBallast water management system according to its operating manual
- Preparation of test waters according to chemical quality criteria
- Preparation of test waters according to biological quality criteria including harvesting and culturing of test organisms

1.2.2 Execution of tests

- Running the PureBallast water management system according to its operating manual
- Monitoring the operational performance of the ballast water management system itself
- Sampling for chemical and biological analyses of initial test waters
- Sampling for chemical, biological and toxicological analyses of treated test waters
- Performing chemical analyses
- Performing biological analyses
- Performing toxicological analyses
- Performing risk characterisation of treated waters

- Preparing for and performing discharge of control water and treated test water to receiving waters

1.2.3 Reporting of results

- Statistical evaluation of the results with regards to the D2 regulation determined by the IMO Convention (IMO, 2004)
- Reporting of the results to the Administration according to the requirements in the IMO Guideline as specified in section 2.4 of Annex 3 (MEPC 53/24/Add.1, 2005)

1.3 Description of test site and technology set-up

1.3.1 Test site

The test will be conducted at NIVA's test site (see Figure 1) located at Solbergstrand 20 km south of Oslo. Sea water is supplied from various depth down to 60 m in the Oslofjord, while fresh water is supplied from ground water bore holes or from a local creek. For full-scale testing, the site include 4 glass-fibre reinforced polyester tanks, supplied with inlet and outlet arrangements and equipment for proper cleaning. Two of the tanks will be covered to prevent light introduction. The tank surfaces will be coated with coatings for ships (Balloxy, Jotun, Norway). Mounted propeller devices with gentle rotation is used to suspend the particles in the test waters evenly and homogenise the contents.

All tanks have a bottom cone to a central drain. The tanks have the following labelling and dimensions:

	Diameter, m	Wall height, m	Total volume, m^3
Prepared test water tank (WST)	12	4	463
Temporary holding tank (TT1)	8	4,5	231
Holding tank for treated water (TT2)	8	4	205
Holding tank for control water (CT2)	8	4	205

For smaller scale testing, circular coated glass fibre tanks (one 50 m^3 and six 16 m^3) can be used. Smaller tanks for prolonged storage of water will be fitted with a light-proof plastic top cover to protect the water from in-falling light.

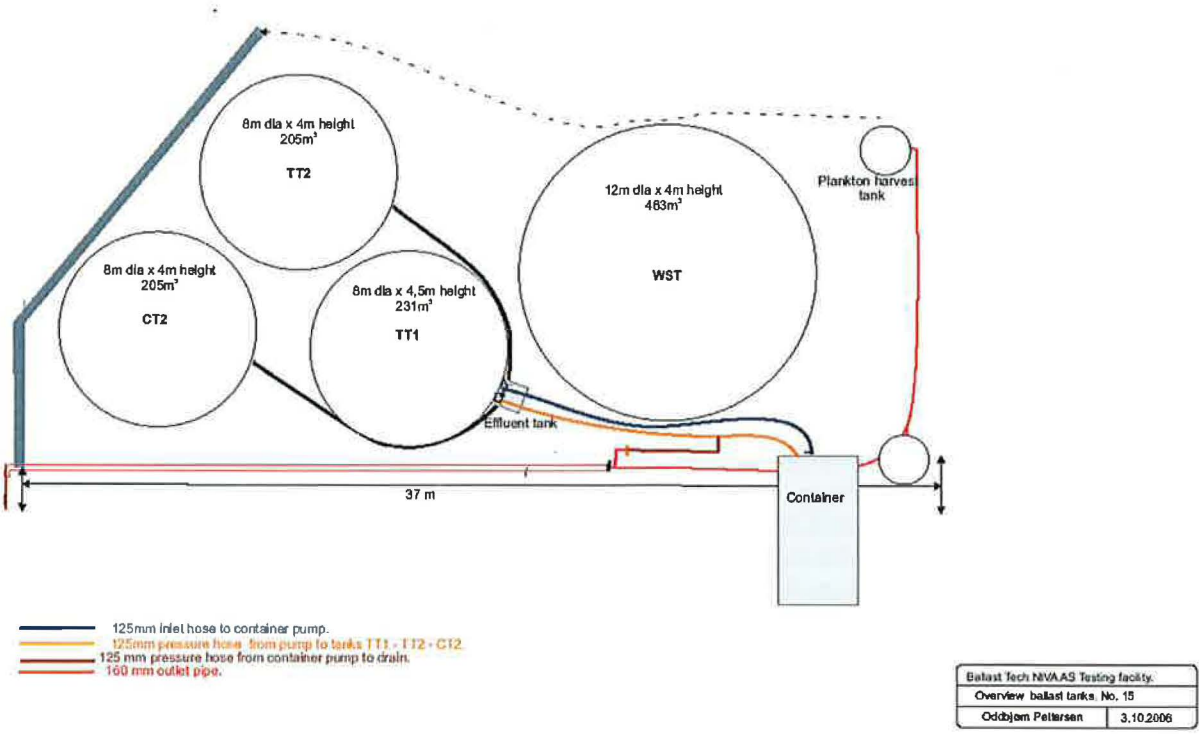

Figure 1 Configuration and placement of the five test tanks at NIVA's test center at Solbergstrand, one with a volume of >400 m^3 and 3 with a volumes >200 m^3 each.

1.3.2 The PureBallast water management system (PureBallast system)

1.3.2.1 Process description

A process description with the chemical features and the discharge characteristics of the Wallenius AOT Process is found in "Appendix L – Process description".

Even though the process is not based on the addition of toxic substances, it can not be excluded that treated ballast water may contain some bio-active reaction products. Therefore, treated ballast water must be tested for toxicity, specifically for:

- Halo-oxy anions
- Chloro-organic compounds
- Bromo-organic compounds
- A general toxicity analysis (oxidized organics)

1.3.2.2 System and component description

The PureBallast system of Alfa Laval is a complete ballast water treatment system composing of three main component ballast water treatment system; 1) a 50 µm filter for removal of larger particles and organisms, 2) the Wallenius AOT (Advanced Oxidation Technology) Module, a patented treatment system using the synergetic effects of *in situ* produced free radicals and direct photo-radiation to inactivate microbes and 3) control and auxiliary equipment including sampling points to control water

flows and measure different alarm levels during operation as well as to take samples during operation and testing.

The system and its components are further described in "Appendix M– Description and the PureBallast system and its components".

1.3.2.3 Physical properties of the PureBallast test equipment

The test equipment is a complete Pure Ballast system with a flow rate of 250 m^3/h. The test system is mounted on two separate racks that are placed on top of each other and are locked with four twist-locks. See Figure 2. The racks are two 20 feet containers with the standard dimensions (o, i = outer, inner measurements):

- $L_o = 6.058$ / $L_i = 5.889$ mm
- $B_o = 2.438$ / $B_i = 2.344$ mm
- $H_o = 2.591$ / $H_i = 2.376$ mm

The weight of the racks is 6 (lower) and 4 (upper) tons respectively.

A centrifugal pump with the same design and properties as most ballast water pumps onboard vessels is mounted in the lower of the two racks. This pump is adaptable for connection to a suction pipe and has an inlet connection of DN 125. The pump has a sucking ability of 4 meters and is possible to be primed through a priming arrangement. In order to protect the pump, a strainer with a length of 300 mm and an outer diameter of 125 mm and with a mesh size of 6 mm is mounted. This strainer corresponds to the strainers found in the sea chest onboard most vessels today.

Figure 2 The PureBallast testsystem

1.4 Organization of the project

The project is a joint cooperation between Alfa Laval/Wallenius Water as the vendor, Norwegian Institute for Water Research (NIVA) as testing site owner and testing organization, and Det Norske Veritas (DNV) as the verification organization.

1.4.1 Presentation of Norwegian Institute for Water Research (NIVA) - testing site owner and testing organization

NIVA is Norway's leading multidisciplinary research institute in the field of use and protection of water. NIVA has more than 80 scientists in the fields of chemistry, biology, limnology, geology, hydrology, environmental technology, environmental toxicology, oceanography, geography, resource management and economy. NIVA has managed several national and international projects related to water and wastewater treatment, ecotoxicology and risk assessment, including testing and verification of ballast water treatment technologies in laboratory and pilot scale. These projects have been funded by The European Community, The Research Council of Norway and private enterprises.

NIVA has well-equipped laboratories for accredited chemical and biological analysis, and ecotoxicological assays, located in Oslo. NIVA is also the owner of Marine Research Station Solbergstrand, an experimental laboratory for large scale experiments and tests, situated at Oslo Fjord.

1.4.2 Quality management plan (QMP) of NIVA

The following documents describe NIVA's quality system:

- Quality manual for NIVA. The quality manual for NIVA is the primary document describing the quality system that is implemented for quality assurance and quality improvements of products and services.

- Quality manual for accredited laboratory analyses and tests. In August 1993 NIVA´s Biological Laboratory was the first laboratory in Norway accredited by Norwegian Accreditation for ecotoxicological tests. At the same time NIVA's Chemical Laboratory, which provides the widest scope of analyses of environmental and water samples in Norway, was accredited according to Norwegian Standard NS-EN ISO/IEC 17025 (Norwegian Accreditation, 1993). NIVA performs annually 150.000 analyses of water quality including nutrients, metals, unspecified organic material, organic micropollutants and test of marine soft-bottom fauna. The accreditation gives the customer a warranty that NIVA´s laboratories use analytical procedures according to internationally acknowledged quality systems (NS-EN ISO/IEC 17025).

- Quality manual for ecological testing based on OECD Principles of Good Laboratory Practice. NIVA now performs ecotoxicity testing according to international standards and is registered as a GLP laboratory in Norway (reg. no GLP 007) and is regularly inspected by the Norwegian Metrology and Accreditation Services. Our ecotoxicological laboratory performs standardised tests (e.g. in accordance with OECD guidelines or ISO standards) as well as special services concerning toxicity, biodegradability and bioaccumulation of chemical substances, products and complex mixtures, e.g. industrial effluents and environmental samples.

- Quality manual for internal control (health, environment and safety). The quality system for internal control (health, environment and safety) is described in; 1) a primary document comprising the principles that are implemented for internal control in health, environment and safety issues, 2) an operative part comprising operating procedures and 3) a documenting part for health, environment and safety related documentation.

- Quality manual for management of projects. A project manual has been developed and has been in use from 2005. The project manual contains checklists for activities and responsibility covering all parts of project accomplishment. One person is independently responsible for quality control of each project.

1.4.3 Presentation of the vendor

Alfa Laval and Wallenius Water have jointly developed and jointly own the PureBallast system. Alfa Laval/Wallenius Water is the vendor in this project.

1.4.3.1 Presentation of Alfa Laval

Alfa Laval is a leading global provider of specialized products and engineered solutions. The equipment, systems and services are dedicated to helping customers to optimize the performance of their processes. Time and time again. Alfa Laval help our customers to heat, cool, separate and transport products such as oil, water, chemicals, beverages, foodstuffs, starch and pharmaceuticals. The worldwide organization works closely with customers in almost 100 countries to help them stay ahead.

Alfa Laval also supplies a number of different products to the marine market such as fuel and lube oil separators, heat exchangers and fresh water generators. The company has in the last couple of years been widening its offering of solutions for environmental protection.

Alfa Laval is certified according to ISO 9000/2000

1.4.3.2 Presentation of Wallenius Water AB

Wallenius Water AB is a privately owned Swedish company located in Stockholm. The company is a wholly owned subsidiary of Wallenius Marine AB, a member of Soya Group. The company was founded in 1996 based on a new and patented technology. This technology is an Advanced Oxidation Technology (AOT) that has been found extremely potent in the treatment of water and removal of all kinds of micro-organisms and bacteria.

Apart from its devotion to the issue of Ballast Water Treatment, Wallenius Water is present with its technology in other areas such as; Animal Husbandry, Horticulture, Cooling Systems, Industrial Process Water as well as other Marine applications. The technology is also widely used in applications for potable water.

1.5 Responsibilities of all project participants

1.5.1 Preparation of test tanks and clarification for the installation of the PureBallast treatment system at the test facility

The test site manager (Oddbjørn Pettersen) will be responsible for the preparation of all test tanks to appropriate condition for conducting tests prior to first test cycle and after each test cycle and for the clarification for the installation of the PureBallast treatment system at the test site.

1.5.2 Installation, clarification, operation and dismantling of the PureBallast treatment system

Installation, clarification, dismantling and operation of the PureBallast treatment system will be executed by the vendor.

1.5.3 Fulfilment of the chemical water quality requirement

Christian Vogelsang (NIVA) will be responsible for the fulfilment of the chemical water quality requirement, including conducting rapid test measurements on site. Tor Gunnar Jantsch (NIVA) will step in for Vogelsang if necessary

1.5.4 Fulfilment of the biological water quality requirement

August Tobiesen (NIVA) and Tor Gunnar Jantsch will be responsible for the fulfilment of the biological water quality requirement, including the cultivation of surrogate organisms; *Brachiomonas submarina*, *Artemia salina*, heterotrophic bacteria. NIVA staff at the test facility (Per Ivar Johannessen) will be responsible for the harvesting of indigenous organisms. Other NIVA personnel will assist in the cultivation of test organisms and serve as "stand-ins" if the responsible person of some reason is unable to attend.

1.5.5 Sample collection and preservation

Tor Gunnar Jantsch and August Tobiesen at NIVA will be responsible for all sampling and preservation of samples connected to the chemical water quality, biological treatment performance and environmental toxicity during all test cycles. Christian Vogelsang will step in for Jantsch if necessary, and Torsten Källqvist/Anne-Marie Bomo will step in for Tobiesen if necessary.

1.5.6 Clarifying test waters for discharge to recipient waters

Tor Gunnar Jantsch will be responsible for the clarification of both treated and non-treated test waters before discharge to the local recipient in the Oslo fjord.

1.5.7 Laboratory chemical measurements

The accredited NIVA laboratory or the accredited AnalyCen laboratory will be responsible for all chemical measurements used to verify the fulfilment of the chemical water quality test criteria: dissolved organic carbon and particulate organic carbon, total suspended solids and salinity. The accreditation warrant that the analyses are conducted according to internationally acknowledged quality systems (NS-EN ISO/IEC 17025) (Norwegian Accreditation 1993).

1.5.8 Measurements of biological treatment factors

August Tobiesen will be responsible for the quantification of organisms >50 µm and ≥10-50 µm. Responsible for bacterial analyses, including heterotrophic bacteria, Coliform group, *E. coli,* enterococcus group and intestinal *Enterococci*, will be carried be Tor Gunnar Jantsch with stand-in and assistance from Åse Bakketun, Anne-Marie Bomo and Christian Vogelsang. If necessary the analysis of heterotrophic bacteria, Coliform group, *E. coli,* enterococcus group and intestinal *Enterococci* can be transferred to an external, qualified laboratory. The analysis of *Vibrio cholerae* and *Vibrio cholerae (serotypes O1 and O139)* will be carried out by the the Norwegian School of Veterinary Science (NVH), Department of Food Safety and Infection Biology. In case of positive identification of *Vibrio cholerae* the sample will be sent to the Norwegian Institute of Public Health, Department of Foodborne Infections for serotyping.

1.5.9 Acute and chronic toxicity tests

Toxicological tests of treated test waters will be carried out by highly qualified personnel according to good laboratory practise (GLP) (reg. no GLP 007) and accredited procedures (e.g. in accordance with OECD guidelines or ISO standards).

1.5.10 Data handling and reporting of test results

The NIVA research manager Helge Liltved will be the main responsible for the reporting of test results to Alfa Laval/Wallenius Water. NIVA researchers Tobiesen, Jantsch, Bomo and Vogelsang will participate in data handling and reporting.

1.5.11 Project management and coordination

Helge Liltved will be the coordinator and manager of the project.

1.5.12 Quality assurance of project

This QAPP will be evaluated by Det Norske Veritas (DNV). NIVA personnel involved with the internal quality assurance of the project will be:

Test water preparation and sampling	Helge Liltved
Laboratory chemical measurements	Håvard Hovin
Measurements of biological treatment factors	Torsten Källqvist
Toxicity testing	Torsten Källqvist
Data handling and reporting	Jarle Nygard

All NIVA staff involved in the practical project work will sign a letter stating that they have carefully read the QAPP and the non-disclosure agreement between Alfa Laval/Wallenius Water and NIVA.

Table 1. Affiliation and responsibilities of all.

Responsibilities	Responsible personnel	Stand-in
Project management	Dr. Helge Liltved	
Site responsible	Dr. Helge Liltved	1. Anne-Marie Bomo
Preparation of test tanks and clarification for the installation of the PureBallast treatment system at the test facility	Oddbjørn Pettersen	1. Per Ivar Johannessen
Installation, clarification, operation and dismantling of the PureBallast treatment system	Carl Tullstedt	1. Alfa Laval/Wallenius Water employees
Fulfilment of the chemical water quality requirement	Dr. Christian Vogelsang	1. Tor Gunnar Jantsch
Fulfilment of the biological water quality requirement – harvesting of indigenous organisms	Techn. ass. Per Ivar Johannessen	1. Oddbjørn Pettersen
Fulfilment of the biological water quality requirement – cultivation of *Brachiomonas submarina* and hatching *Artemia salina*	Dr. August Tobiesen	1. Torsten Källqvist 2. Anne-Marie Bomo
Fulfilment of the biological water quality requirement – cultivation of heterotrophic bacteria	Dr. Tor Gunnar Jantsch	1. Åse Bakketun 2. Christian Vogelsang
Sample collection and preservation – chemical water quality	Dr. Tor Gunnar Jantsch	1. Christian Vogelsang
Sample collection and preservation – organisms ≥50 µm and ≥10-50 µm	Dr. August Tobiesen	1. Torsten Källqvist 2. Anne-Marie Bomo
Sample collection and preservation – bacteriology	Dr. Tor Gunnar Jantsch	1. Christian Vogelsang 2. Anne-Marie Bomo
Clarifying test waters for discharge to recipient waters	Dr. Tor Gunnar Jantsch	1. August Tobiesen 2. Oddbjørn Pettersen
Laboratory chemical measurements	M. Sc. Håvard Hovind on behalf of accredited personnel at the NIVA lab	1. AnalyCen
Measurements of biological treatment factors – organisms ≥50 µm and ≥10-50 µm	Dr. August Tobiesen	1. Anne-Marie Bomo
Measurements of biological treatment factors – heterotrophic bacteria, Coliform group, *E. coli,* enterococcus group and intestinal *Enterococci*	Dr. Tor Gunnar Jantsch Techn. ass. Åse Bakketun	1. Anne-Marie Bomo 2. Christian Vogelsang 3. AnalyCen
Measurements of biological treatment factors – *Vibrio cholerae*	Norwegian School of Veterinary Science (NVH), Department of Food Safety Per Einar Granum	
Measurements of biological treatment factors – serotyping of *Vibrio cholerae (serotypes O1 and O139)*	Norwegian Institute of Public Health, Department of Foodborne Infections	
Acute and chronic toxicity tests	Dr. August Tobiesen Res. ass. Sigurd Øksnevad	1. Torsten Källqvist
Data handling and reporting of test results	Dr. Helge Liltved Participants: August Tobiesen, Tor Gunnar Jantsch, Anne-Marie Bomo, Christian Vogelsang	
Quality assurance of project – • QAPP • Test water preparation and sampling • Laboratory chemical measurements • Measurements of biological treatment factors • Toxicity testing • Data handling and reporting	• Hanna Lee Behrens • Dr. Helge Liltved • M. Sc. Håvard Hovin • M.Sc. Torsten Källqvist • M.Sc. Torsten Källqvist • Jarle Nygard	1. Egil Dragsund

2. Experimental approach

2.1 Technology installation and shakedown procedures

General
The manual shall always be available during operation of the system (Alfa Laval, 2005). More information together with a system drawing is available in "Appendix M – Description and the PureBallast system and its components".

Power Connections

- Turn on the power switches for 380 and 220 VAC inside the main fuse cabinet.
- Connect three-phase, without zero, fused with 200 A to the plinth in the pump connection cabinet.
- Check the phase connection by starting the pump – the pump should rotate clockwise.
- Connect three-phase, without zero, fused with 32 A for the rest of the equipments connection cabinet, plinth connection.
- Check the phase connection by starting the compressor – remove the air filter on the compressors air inlet on the backside of the beige hood and check that the fan is rotating clockwise).

Compressed air

- The rotation direction on the compressor is checked according to the description above for power connections.
- Check that the cooling air for the compressor is not blocked. The inlet is from the backside and the outlet is on the top of the hood.
- Check that the compressor panel indication light show green light for correct power feeding.
- Check the compressors coolant/lubricating oil – level, the sight glass should be full when the compressor is stopped. The sight glass is placed under the black hood to the right, the hood is locked with two twist locks – when refilling use:
- SSR ULTRA-PLUS coolant.
- Check that the lamp panel is indicating ready to start on the compressor.

Pump
The rotation direction is checked according to the description above (power connections) and note that this can not be done when the pump is dry.

2.2 Technology start-up and stop procedures

2.2.1 System start-up procedures

Air-dryer

- The air-dryer should start at least 5 minutes before the compressor and it should always be 3 minutes between stop and restart of the air-dryer.
- The inlet valve opens slowly and then the outlet valve. The by-pass valve should be closed.
- Check that it drips from the draining to ensure that is not blocked.
- Check that the indication does not show "RED"

Compressor

- The compressor is started by the left button on the panel.
- Drain possible condenses water from the drain valve on the tank for compressed air.
- Open the tanks outlet valve and check that the outgoing pressure is around 7 bar.

Compressed air filter
Check that the indication does not show "RED".

Pump
With a static pressure on the pumps suction side you only need to vent the pump house – Open suction valve V201-41 and vent the pump and system. The pump is started with a closed outlet valve V201-28. After that should the outlet valve opens slowly until the right flow is reached (check on the flow meters display).

When the pump is to be started up with air on the suction side is it necessary to help the pump to get started – close suction valve V201-41 and via valve V201-35 is water supplied from an external source and vent the pump and system to the same level as the AOT unit. The pump is started with a closed outlet valve V201-28. After that should the outlet valve and suction valve V201-41 opens slowly at the same time and priming valve V201-35 closes slowly until the right flow is reached (check on the flow meters display).
With this method are you helping the pump to get started and to create the necessary under pressure on the pumps suction side. If you stop/start many times can you leave the priming valve open and supply with water to avoid problems when re-starting.

When recalculating from a tank shall the tanks volume not be less then 3 m^3, to ensure that the temperature is too high. It is important to check the temperature continuously.

Pre-Filter
When the filter is pressurized shall it be vented by the ball valve on the top and then activated by the "Test" button to be ready to start.

2.2.2 System stop procedures

Pump
Close valve V201-28 and thereafter stop the pump. If the pump is not to be used for a longer period or if it there is a risk of frost, the pump and the complete system should be drained.

Compressor
The compressor is stopped by pressing the button to the right "Normal stop" on the panel.
Drain condenses from the compressed air tank by using the bottom valve and closes the tanks outlet valve.

Air-Dryer
Must always be stopped at least 2 minutes after the compressor, it should always be at least 3 minutes between stop and restart of the air-dryer.
The inlet and outlet valve should be closed.
The air-dryer can never be supplied with compressed air when it is shut off.

Power Connections
Turn off the 380 V switch and cut the 220 V switch in the fuse cabinet.

2.3 Technology calibration checks

There are a number of parameters that are very important for satisfactory operation of the PureBallast system. Most of these parameters such as lamp operation, valve positions and pressure levels are controlled by the control system and if no alarms are given, the system is operating satisfactory. The flow rate of the system is very important and is constantly logged by the control system. After each test run, a log from the control system will accomplish the data report from each test run.

2.4 Preparation of test waters

Test waters will be prepared in a 400 m^3 tank (WST) from high salinity sea water from 60 meters depth or from brackish surface water depending on the required salinity (>32 PSU or 3-32 PSU, respectively, with a minimum difference of 10 PSU). The 400 m^3 of test water will be use for both testing and control. A combination of harvested indigenous organisms and cultured surrogate species (>50 µm: *Artemia salina*; 10-50 µm: *Tetraselmis suecica* and/or *Brachiomonas submarina;* if necessary heterotrophic bacteria) will be added to fulfil the biological water quality criteria (see Table 2), and freshwater, soluble lignin, crystalline cellulose and kaolin will be added to adjust the salinity and the contents of dissolved organic carbon (DOC), particulate organic carbon (POC) and total suspended solids (TSS), respectively, to within the limits of chemical water quality criteria (see Table 3). Test waters 1 and 2 in Table 3 will be used.

Table 2. Required biological water quality in influent test water and in control after 5 days storage.

Organism group	Influent water	In control after 5 days storage
≥50 µm min. dimension	Pref. 10^6 m^{-3}, ≥10^5 m^{-3} Min. 5 species from 3 diff. phyla/divisions	> 10x <10 viable organisms per m^3
≥10-50 µm min. dimension	10^4 ml^{-1}, ≥10^3 ml^{-1} Min. 5 species from 3 diff. phyla/divisions	> 10x <10 viable organisms per m^3
Heterotrophic bacteria	≥10^4 ml^{-1}	

Table 3. Required chemical water quality of test waters before any additional test organisms have been added. Salinities should be separated by at least 10 PSU.

	Salinity	DOC	POC	TSS
Test water 1	>32 PSU	>1 mg/l	>1 mg/l	>1 mg/l
Test water 2	3-32 PSU	>5 mg/l	>5 mg/l	>50 mg/l
Test water 3	≤ 3 PSU	>5 mg/l	>5 mg/l	>50 mg/l

2.4.1 Assurance of fulfilment of chemical water quality test criteria

The water quality with regards to chemical criteria will be prepared several days prior to addition of organisms and treatment of the water in the treatment system. The 400 m^3 tank will be filled with water from the appropriate source. Rapid quantification test kits will be used to estimate the salinity and concentrations of DOC, POC and TSS in the test waters on site. The amount of soluble lignin, crystalline cellulose and kaolin to be added to the test waters will be calculated from measured values and the known concentrations of the respective stock solutions, and the final concentrations will be checked by repeated measurements on site. Additionally samples will be taken and analysed by standard methods for DOC, TOC (POC) and TSS at an accredit laboratory. The samples will be taken from the middle of the water column in the tank. Before sampling, all water contents will be homogenized as described in chapter 3.1

Any necessary adjustment to the chemical water quality will be made before any additional organisms are added to the test water to minimize stress on the organisms

2.4.2 Assurance of fulfilment of biological water quality test criteria

The concentrations and variety within all organism groups in Table 2 is usually very low in deep sea water, hence, organisms have to be added to fulfil the criteria. The minimum concentration criteria will also be difficult to fulfil for most groups (except the heterotrophic bacteria group) in surface water by the usual water quality at Solbergstrand. The criteria for minimum concentration in the control after 5 days storage may make it necessary to add heterotrophic bacteria to the inlet test water.

2.4.2.1 Harvesting of indigenous organisms

Harvesting of indigenous algae and planktonic animal species, using a Unik Filter type 450 (Unik Filtersystems, Os, Norway) equipped with a 20 µm mesh size screen, is done to assure the fulfilment of the criteria regarding at least 5 species from 3 different phyla/divisions of both test groups of organisms ≥10 µm in minimum dimensions. The harvesting process has been shown to be relatively gentle to the organisms; surface water (1 m depth) is smoothly pumped (ca. 3 m^3/h) by a jet-pump to the inlet side of the screen, and algae and animals are washed from the screen to a collecting tray (ca. 100 l/h, e.g. approx. 30x conc.) and transported to a storage tank. The transport to the storage tank and from the storage tank to test-tanks will be as gentle as possible with a minimum of pumping. The variability (phyla, species), viability and general conditions of harvested organisms will be evaluated by microscopy to ensure that the test criteria most probably will be fulfilled.

2.4.2.2 Cultivation of Artemia salina and Tetraselmis suecia and/or Brachiomonas submarina

The criteria of minimum concentration of organisms ≥50 µm in minimum dimension is fulfilled by adding cultured *Artemia salina*, and the equivalent criteria for organisms ≥10-50 µm in minimum dimension is fulfilled by adding cultured *T. suecica* and/or B. *submarina*.

A. *salina*: Resting cysts of *Artemia salina* are available commercially. Hatching of cysts are achieved by adding approximately 0.1 g of cysts to 1 litre of 20 ‰ salinity seawater. The culture is incubated with a bright light source at a temperature of 22-26 °C with good aeration in the medium. Full hatching is usually achieved in about 48 hours. It is possible to hatch approximately 100 000 nauplii per litre. *Artemia* nauplii are hatched with a supply of food (egg yolk) and will therefore live for up to a week without any external food supply. Survival length is twice that if the naupli is stored at 8-10 °C. However, nauplii should be used within 2-3 days in order to achieve high viability.

T. suecica and/or B. submarina: *The algae* are grown autotrophically in a seawater growth media with added nutrients. Large volume cultures need gentle aeration in order to maintain oxygen levels. We have been able to reach densities close to 10^9 per 100 ml.

The necessary cultivation volume is calculated based on a final density (10^5 nauplii per litre for *A. salina* and 10^7 per ml for *T. suecica and/or B. submarina*, the final volume of the test water 400 m^3) and the desired concentration of the organism in the test water (10^5 per m^3 for *A. salina* and 10^3 per ml for *T. suecica and/or B. submarina*).

A critical point is the survival of *T. suecica and/or B. submarina* during the 5 days storage in the control water rich in grazing organisms. To estimate and verify the survival of *T. suecica and/or B. submarina* in the control without grazers present, a specified volume of the control water will be sieved through a 50 µm mesh to remove the majority of grazing organisms and stored in a separate container within the control water tank at the same temperature as the rest of the water and in darkness.

2.4.2.3 Bacteria

The concentration of heterotrophic bacteria in the surface water is normally exceeding the required ≥10^4 ml^{-1}, while the heterotrophic bacteria criteria for the high salinity test water is expected to be fulfilled by the heterotrophic communities accompanying the cultured *Tetraselmis suecica and Brachiomonas submarina* and *Artemia salina*, and, if necessary, added as heterotrophic bacteria.

2.4.3 Discharge of waters

The potential environmental impact of discharge of test and control waters will be evaluated and in the case of adverse effects measures taken to counteract the adverse effects by treating the waters prior to discharge. Discharge permit from the Norwegian Pollution Control Authority represented by the County Governor in Oslo and Akershus has been obtained.

2.5 Running test cycles

The different water transfers between tanks via the PureBallast system during a test cycle including the control is shown in Figure 3. One test cycle (blue lines) involves consecutive treatment of 200 m^3 test water by the 50 µm filter unit and the Wallenius AOT Module of the PureBallast system transferring the test water from tank with prepared test water (WST) to a temporary holding tank (TT1) before a second treatment by the Wallenius AOT unit and transfer to a storage tank (TT2) for at least five days storage. A control cycle (red lines) is run by transferring 200 m^3 of the same type of prepared test water from WST to a temporary holding tank (TT1) using the pump of the PureBallast system, but in by-pass of the treatment units before transfer to a second storage tank for at least five days storage (CT2). One cycle including one control will be run for each of the test waters (high and medium salinity).

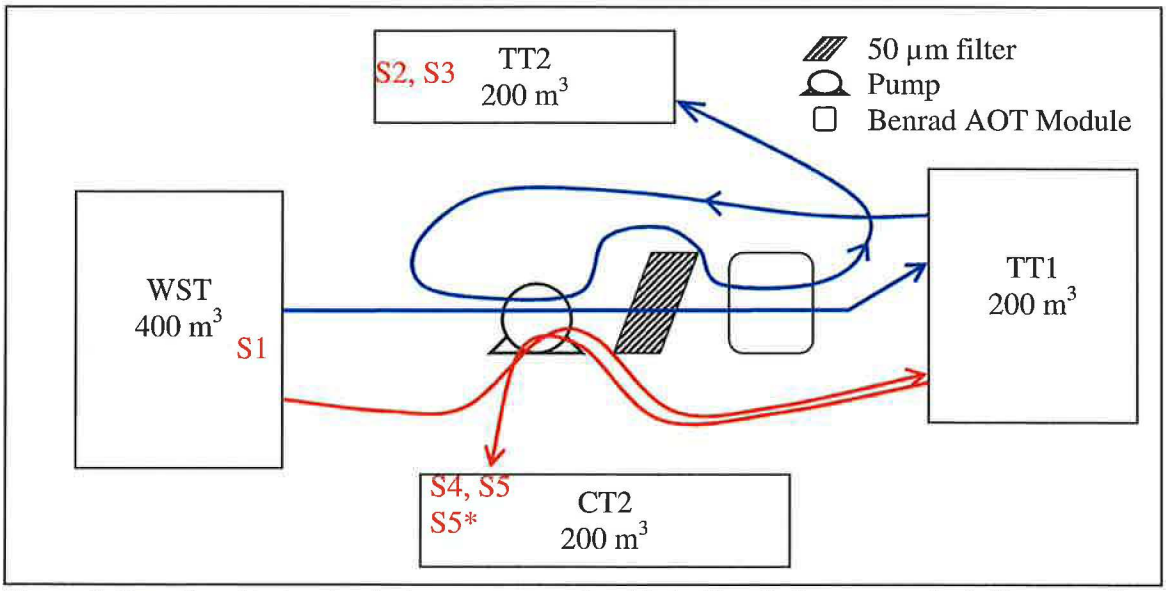

Figure 3. Transfer of test water during one test cycle with the PureBallast system including a test line (blue) and a control line (red). Sampling numbers are indicated by S1-S5.

2.6 Sampling and measurements

Table 4 summarizes all types of measurements to be taken during the study. Samples are withdrawn to document:

1) that the water quality in the initial test water (WST) is meeting the chemical and biological water quality criteria defined by IMO,
2) the efficiency of the Alfa Laval PureBallast system in removing/inactivating target organisms (TT2) immediately and after 5 days storage,
3) the water quality in the control tank (CT2) as a reference to the water quality in TT2,
4) the chronic and sub-chronic environmental toxicity of the water directly after treatment.

Sampling numbers are indicated by S1-S5 (S5*) in Figure 3. All grab samples (see Table 4) will be collected in triplicates. S1 will be collected in WST directly before treatment, S2 will be collected in TT2 immediately after treatment and S3 after at least 5 days storage in TT2. The same procedure will be used when sampling from the control line (S4, S5 and S5*). The sample marked with an asterix will be taken from the volume of control water separated from the grazing organisms after 5 days storage. Procedures for sampling and transfer are described in detail in section 3.

Table 4. Parameters to be measured during the study; time and type of sampling and measurement location.

Parameter	Sample number	Type of sample	Location
Operational parameters for the PureBallast treatment system			
Chemical water quality measurements			
Temperature	S1, S2, S3, S4, S5, S5*	*In situ*, continuous	Solbergstrand
pH	S1, S2, S3, S4, S5, S5*	*In situ*, continuous	Solbergstrand
Dissolved oxygen	S1, S2, S3, S4, S5, S5*	*In situ*, continuous	Solbergstrand
Salinity	S1, S2, S3, S4, S5	*In situ*, continuous	Solbergstrand
Dissolved organic carbon (DOC)	S1, S2, S3, S4, S5	Discrete grab	Solbergstrand, NIVA Oslo
Particulate organic carbon (POC)	S1, S2, S3, S4, S5	Discrete grab	Solbergstrand, NIVA Oslo
Total suspended solids (TSS)	S1, S2, S3, S4, S5	Discrete grab	Solbergstrand, NIVA Oslo
Biological treatment performance parameters			
Organisms > 50 µm	S1, S2, S3, S4, S5	Discrete grab	Solbergstrand
Organisms ≥10-50 µm	S1, S2, S3, S4, S5, S5*	Discrete grab	NIVA Oslo
Heterotrophic bacteria	S1, S2, S3, S4, S5	Discrete grab	NIVA Oslo
Coliform bacteria	S1, S2, S3, S4, S5	Discrete grab	NIVA Oslo
E. coli	S3	Discrete grab	NIVA Oslo
Enterococcus group bacteria	S1, S2, S3, S4, S5	Discrete grab	NIVA Oslo
Intestinal *Enterococcus*	S3	Discrete grab	NIVA Oslo
Vibrio cholerae	S1, S2, S3, S4, S5	Discrete grab	NIVA Oslo
Vibrio cholerae (Serotype O1 and O139	S3	Discrete grab	NIVA Oslo
Environmental toxicity evaluation parameters			
Acute tests			
Acute toxicity; growth inhibition of green algae	S2, S4	Discrete grab	NIVA Oslo
Acute toxicity on marine copepods	S2, S4	Discrete grab	NIVA Oslo
Acute toxicity on fish test	S2, S4	Discrete grab	NIVA Oslo
Chronic tests			
Toxicity; growth inhibition of green algae	S2, S4	Discrete grab	NIVA Oslo
Toxicity; reproduction of marine copepods	S2, S4	Discrete grab	NIVA Oslo
Toxicity; juvenile fish growth test	S2, S4	Discrete grab	NIVA Oslo

2.7 Time schedule for the testing period

The time schedule of the testing period is shown in table 5. The time schedule does not allocate time to unforeseen situations. Such situations may occur, and a delay of 3-4 weeks should be accepted.

Table 5. Time schedule for testing

	Week no																			
TASK NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	39	40	41	42	43	44	45	46	47	48	49	50	51	52	1	2	3	4		
Cultivation of algae	X	X	X	X	X	X	X	X	X	X	X	X								
Cultivation of Artemia		X	X	X	X	X	X	X	X	X	X	X								
Cultivation of bacteria		X	X	X	X	X	X	X	X	X	X	X								
Preparation of test water			X		X		X		X		X									
Technology installation	X	X																		
Water quality 1 and 2																				
Test cycle 1			X					X												
Test cycle 2				X					X											
Test cycle 3					X					X										
Test cycle 4						X					X									
Test cycle 5							X					X								
Toxicity testing			X	X	X	X	X	X												
Toxicity reporting					X	X	X	X	X											
Quality assurance	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Data reduction/validation								X	X	X	X	X	X	X	X	X	X			
Reporting									X	X	X	X	X	X	X	X	X			

3. Sampling procedures

3.1 Representativeness of samples

Before any samples are collected the following procedure is carried out to assure that representative samples may be withdrawn:

1. The water is homogenized using a propeller device before sampling from WST and from TT2 and CT2 after 5 days storage. The turbidity in different sections of each tank (upper part, middle part and bottom part, in both periphery and mid section) is then measured by a handheld submersible probe (YSI – 600 OMS) during homogenization. When any turbidity measurement is within a 10 % deviation from the average turbidity of all measurements in the tank, sampling is started.
2. The particulate content in TT1 is kept in suspension between transfer from WST and transfer to TT2 and CT2, respectively, by using propeller devices. The particulate content in TT2 and CT2 is kept in suspension after transfer from TT1, using propeller devices. The turbidity in different sections of each tank (upper part, middle part and bottom part, in both periphery and mid section) is then measured by a handheld submersible probe (YSI – 600 OMS) during homogenization. When any turbidity measurement is within a 10 % deviation from the average turbidity of all measurements in the tank, sampling is started.

3.2 Sampling of test waters

Table 6 summarize which sampling equipment is used to collect samples for the individual parameters.

Table 6. Equipment and containers used for sampling and necessary and sampled volume for the individual parameters.

Parameter	Sampling equipment	Sample container	Collected volume WST, CT2	Collected volume TT2
Turbidity	Turbidimeter submersible probe	-	-	-
Temperature	Temp. meter	-	-	-
pH	pH probe	-	-	-
Dissolved oxygen	DO probe	-	-	-
Salinity	Probe	-	-	-
DOC	Directly	Acid washed glass bottle	100 ml	100 ml
POC	Directly	Acid washed glass bottle	100 ml	100 ml
TSS	Directly	Clean plastic bottle	1000 ml	1000 ml
Organisms ≥ 50 µm	Sieving*		20 l	1 m^3
Organisms 10-50 µm	Directly	Clean glass bottle	1000 ml	10 l
Heterotrophic bacteria	Directly	Sterile glass bottle	1000 ml	1000 ml
Coliform bacteria				
Enterococcus group bacteria				
Vibrio sp.				
Vibrio cholerae				

* A 20-litre grab sample (from WST and CT2) or 1 m^3 collected through a siphon spillway (from TT2) is concentrated to a volume of 40-100 ml through a plankton net with diagonal dimensions of 50 µm.

The procedures for collecting samples from the different tanks and sampling times are as follows:

1) *Sampling of organisms ≥ 50 µm in WST (S1) and CT2 (S4 and S5).* A plastic bucket is used to collect a 20 litre sample. The sampled water is slowly sieved through a plankton net (50 µm diagonal dimensions) attached to a plastic cup, collecting the organisms in the cup.
2) *Sampling of organisms ≥ 50 µm in TT2 (S2 and S3):* A siphon spillway is used to collect 3x 1 m^3 test water from TT2 directly after transfer from TT1 and after 5 days storage. The water is sieved directly through a plankton net (50 µm diagonal dimensions) attached to a plastic cup, collecting the organisms in the cup. The sieved water is collected in known volume tanks to ensure accurate sampling volume.
3) *Sampling of organisms 10-50 µm in WST (S1), TT2 (S2 and S3) and CT2 (S4, S5 and S5*):* Organisms with a minimum diameter between 10 µm and 50 µm are sampled as 1000 ml for control and 10 l for treated water grab samples with a clean glass bottle.
4) *Sampling of bacteria in WST (S1), TT2 (S2 and S3) and CT2 (S4 and S5):* Bacterial samples are collected as 1000 ml grab samples by slowly submerging a 1000-ml sterile glass bottle. The bottle is closed immediately after sampling.

3.3 Sample preservation

Preservation methods and expected storage/holding times before measurement are shown in Table 7.

Table 7. Preservation methods and expected storage/holding times before measurement (ISO/CD 5667-3, 2001).

Parameter	Preservation	Maximum holding time	Expected storage time
Temperature	-	-	0
pH	-	-	0
Dissolved oxygen	-	-	0
Salinity	-	-	0
Dissolved organic carbon (DOC)	Acidify with 1 ml 4 M H_2SO_4 per 100 ml (pH<2), 4°C	7 days	0-5 days
Particulate organic carbon (POC)	Acidify with 1 ml 4 M H_2SO_4 per 100 ml(pH<2), 4°C	7 days	0-5 days
Total suspended solids (TSS)	4°C	24 hours	<24 hours
Organisms ≥ 50 µm	4°C	6 hours	< 2 h
Organisms 10-50 µm	4°C	24 hours	< 24 h
Heterotrophic bacteria	4°C	24 hours	< 24 h
Coliform bacteria			
Enterococcus group bacteria			
Vibrio sp.			
Vibrio cholerae			

3.4 Measures to avoid cross-contamination during test water transfer and sampling

To avoid cross-contamination between consecutive test waters upon transfer between tanks all pipelines are flushed for 2-3 min with sea water from 60 meters depth or ground water with

documented quality between each test cycle, followed by rinsing with high temperature water (80-90°C).

To avoid cross-contamination during sampling the propeller, buckets, siphon overflow and plankton net are thoroughly rinsed in sea water from 60 meters or ground water between each sampling.

4. Testing and measurement protocols

An overview of all analytical measurements with instruments used, references and current uncertainty of the method applied is shown in Table 8 (chemical) and Table 9 (biological).

4.1 In situ measurements

4.1.1 Temperature

Temperature is measured *in situ* using a calibrated thermometer. Temperature is reported in °C.

4.1.2 pH

pH is measured *in situ* using a calibrated probe and pH-meter.

4.1.3 Dissolved oxygen (DO)

Dissolved oxygen (DO) is measured *in situ* using a calibrated probe and meter. DO is reported as mg O/l.

4.1.4 Salinity

Salinity is measured *in situ* using a calibrated salioterm. Salinity is reported in PSU or in ‰. Salinity will also be measured at the NIVA laboratory in Oslo using an accredited method (see Table 8).

4.2 Discrete samples

4.2.1 Dissolved organic carbon (DOC)

DOC is measured by an accredited method based on Norwegian Standard NS-ISO 8245 (NIVA method G5-1) at NIVA after filtering the sample through a GF/F filter (0.7 µm). The sample is acidified with phosphoric acid and aerated with oxygen to remove inorganic carbon (NB! Removes also volatile organic carbon). The sample is injected in a quartz tube filled with a platinum catalyser at 680 °C. The organic carbon compounds are oxidized to CO2 which is quantified using an NDIR detector (Phoenix 8000 TOC-TC analyser with sample carousel STS 8000) with oxygen as carrier. Detection limit is 0.5 mg C/l.

4.2.2 Particulate organic carbon (POC)

POC is calculated as the difference between the level of total organic carbon in the sample, measured on the non-filtered sample (see 4.2.1), and the measured DOC level of the same sample.

4.2.3 Total suspended solids (TSS)

A nucleopore capillary filter (0,4 µm) is dried at 40-50 °C for 2 hours and the tara is determined by weighing on a micro weight (Sartorius 4503 Micro) equipped with an ion source (Static Eliminator Bar Pu 210 Item no. LC 9793) removing static electricity. The sample is filtered through the filter, which again is dried at 40-50 °C for 2 hours before it is weighed on the micro weight. The TSS is represented by the weight increase. Lowest reported value: 0.1 mg/l. Ed. 2540 D pp 2-54: Total

suspended dried at 103 – 105 °C. The method used is according to Standard Methods (APHA, 1995), but modified using nucleopore capillary filter in stead of glass fibre filter.

4.2.4 Organisms >50 µm

Organisms >50 µm are inspected in microscope at 10-40x magnification within 6 hours of sampling. Viable organisms are counted and identified based on motility and integrity according to OECD (1985): OECD Test Guideline for Testing of Chemicals 202, "*Daphnia* sp. acute immobilisation test and reproduction test".

4.2.5 Organisms ≥10-50 µm

CFDA-staining method

The viability of the micro-plankton (>10-50 µm) is determined by observing cells incubated with 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) according to Ganassin et al. (2000). A 10 ml sample is incubated for 1 hour with 4 µmol of CFDA-AM. The sample is fixed with formalin and filtered onto black polycarbonate filters (25 mm). The filter is mounted on a glass slide in paraffin oil and frozen. CFDA-AM is hydrolysed only in a living cell. CFDA-AM is a marker for cell membrane integrity and may be measured directly in cells. In principle, the non-fluorescent chemicals CFDA-AM is taken up in the cytosol, where it becomes hydrolysed into fluorescent end products. These end products are trapped inside the cellular compartment and may be observed in an epifluorescence microscope using excitation filter 485 nm and emission filter of 530 nm. In the epifluorescence microscope viable cells are observable as brightly yellow/green coloured cells, while non viable cells are pale green (heterotrophic cells) or pale green with red autofluorescence of the chloroplast (photoautotrophs). Numbers of viable and non viable cells are counted at a magnification of 300-480 times.

Dilution-culture method

The dilution-culture method is used as a complementary method for testing viability of organisms ≥10-50 µm. The method used is based on Throndsen (1978). Briefly, the dilution series is achieved by adding 1 ml sample to 9 ml of algal growth media (20% Z8 seawater media). After gentle but thoroughly mixing, 1 ml of this sample is further diluted with 9 ml of growth medium. In this way, a series of 10x dilution series are made. Number of dilution steps is set according to the expected cell density on the original sample. Five parallels are recommended to provide statistical significance of estimated number. The test-tubes are sealed/corked and incubated in a room or cabinet with controlled light and temperature conditions. After two to three weeks (depending on light and temperature conditions) the cultures are examined microscopically and the presence of each species in the tubes is noted. When the growth pattern (presence or absence) of each species through the culture series has been determined, the most probable number (MPN) per unit volume can be estimated from tables. Tables are given in Throndsen (1978).

4.2.6 Heterotrophic bacteria

Heterotrophic bacteria are quantified according to a modified version of Norwegian Standard NS-EN 6222/NS 4791 using a marine agar for isolation of marine heterotrophic bacteria.

Table 8. Summary of all chemical measurements.

Parameter	Units	Instrument	NIVA method	Reference	Detection limit	Uncertainty			
						Control	#	Average	Std
***In situ* measurements**									
Temperature	°C		-	Instrument manual	0				
pH	-		-	Instrument manual	-				
Dissolved oxygen	mg O/l		-	Instrument manual	0.0				
Salinity	PSU, ‰	Manually	-	Instrument manual					
Discrete samples									
Salinity	PSU, ‰	Autosal model 8400A	A 3	UNESCO (1981)	0.005	Standard seawater IAPSO: 34,99252 PSU	9	34.9934	0.00105
DOC	mg C/l	Apollo 9000 HS catalytic oxid. 680°C	G5-3	Norwegian Standard NS-ISO 8245	0.2	5.0 mg C/l, KHftalat	20	4.97	0.10
POC	mg C/l		G5-3						
TSS	mg/l	Sartorius 4503 Micro weight	B4	Standard methods (1995)	0.1	Double analysis natural sample, TSM > 2 mg/l	32	0.9 % difference	11.1%

Table 9. Summary of all biological measurements.

Parameter	Units	Instrument	NIVA method	Reference	Detection limit
Organisms > 50 µm	#/m^3	Dissecting microscope 10-40x magnification	K 9	OECD Test Guideline (1985)	1/m^3
Organisms 10-50 µm	#/ml	Epifluorescence microscope (excitation filter 485 nm; emission filter 530 nm) at 300-480 times magnification	-	Ganassin *et al.* (2000)	1/ml
Organisms 10-50 µm	#/ml	Serial dilution technique	-	Throndsen (1978)	0,2/ml
Heterotrophic bacteria	cfu/ml	Water Plate Agar CM1012 (Oxoid), directly or after concentration by filtration	J 3	Norwegian Standard NS-EN 6222/NS 4791	1 cfu/ml
Coliform	cfu/100 ml	m-Endo Broth MF 274930 (Difco) after concentration by filtration	J2	Norwegian Standard NS 4788	1 cfu/100 ml
E. coli	cfu/100 ml	m-FC agar after concentration by filtration, 24 h, 44 °C, produce acid from lactose, presumptive *E.coli* produce gas from lactose or manitol and indol from tryptophan, 24 h, 44 °C.	-	NS 4792 or NS-EN ISO 9308-3	1 cfu/100 ml
Enterococcus group	cfu/100 ml	m Enterococcus Agar 274620 (Difco) after concentration by filtration	J6	Norwegian Standard NS-EN ISO 7899-2	1 cfu/100 ml
Intestinal *Enterococci*	cfu/100 ml	Specific agar, after concentration by filtration 44 °C, , verification on bile-esculinagar as coloured colonies	-	NS-EN ISO 7899-2 or 7899-1	1 cfu/100 ml
Vibrio cholerae	cfu/100 ml	T.C.B.S. Cholera-medium Agar CM0333 (Oxoid) after concentration by filtration	-	APHA (1995), terminating the method after determining total count of *Vibrio* and prior to specific identification of *Vibrio cholerae*	1 cfu/100 ml?
Vibrio cholerae (serotypes O1 and O139)	cfu/100 ml	T.C.B.S. Cholera-medium Agar CM0333 (Oxoid) after concentration by filtration, PCR, Serotyping	-	APHA (1995), terminating the method after determining total count of *Vibrio* and prior to specific identification of *Vibrio cholerae*	1 cfu/100 ml

4.2.7 Coliforms

Coliform bacteria are quantified according to Norwegian Standard NS 4788 at a temperature of 27±0.2 °C and an incubation period of 18-24 hours.

4.2.8 *E.coli*

E. coli are quantified according to Norwegian Standard NS 4792 or NS-EN ISO 9308-3 at a temperature of 44±0.2 °C and an incubation period of 24 hours.

4.2.9 Enterococcus group

Total fecal *Enterococci* are quantified according to Norwegian Standard NS-EN ISO 7899-2 at a temperature of 37±0.2 °C and an incubation period of 44 hours.

4.2.10 Intestinal *Enterococci*

Intestinal *Enterococci* are quantified according to Norwegian Standard NS-EN ISO 7899-2 or 7899-1 at a temperature of 44±0.2 °C and an incubation period of 44 hours.

4.2.11 *Vibrio cholerae*

Vibrio cholerae are quantified according to the method described by the American Public Health Association (APHA,1995). The total number of *Vibrio* sp., are determined by filtering of a 1-100 ml sample, the filter is placed on TCBS Cholera-medium agar plates (manufacturer: Oxoid), incubated at 35 °C, and the colonies are counted after 3 to 4 days.

4.2.12 *Vibrio cholerae* (Serotypes O1 and O139)

Vibrio cholerae identified above are confirmed for each colony from above by PCR and serotyping with specific antibodies for the outer antigens O1 and O139.

4.3 Chemical measurements – initial quick tests

During preparation of test waters commercially available test kits are used to get rapid estimates of DOC, POC and TSS levels to the check and substantiate that the chemical water quality criteria are met. These results will be verified by the accredited laboratory measurements.

Before sampling and transfer between tanks the homogeneity of suspended solids in the whole water body is checked by measuring the turbidity at different levels in the tank.

4.3.1 Dissolved organic carbon (DOC)

The sample is filtered through a 0.45 µm membrane filter (Millipore) and the organic content is measured colorimetrically by the HACH method 10129 for samples with a DOC content within the range 0.3-20.0 mg C/l.

Procedure:

1) 0.4 ml buffer solution of pH 2.0 (HACH, 452-33) is added to 10 ml sample and mixed for 10 min. The pH is checked using a pH paper (pH 2.0).
2) The content of one Persulfate Powder Pillow is added to each of two Low Range Acid Digestion vials (27603-45)
3) 3.0 ml organic-free water is added to one of the vials (reagent blank) and 3.0 ml prepared sample to the other vial (sample).
4) Two blue Indicator Ampules are rinsed with deionised water and wiped with a soft, lint-free wipe. One Indicator Ampoule is lowered into each vial and snapped open when the score mark on the ampoule is level with the top of the vial.
5) The vials are capped and placed in a COD reactor for 2 hours at 103-105 °C.

6) The vials are removed from the reactor and cooled for one hour before measurement at 430 nm and 598 nm using HACH program 427 Organic carbon LR on a HACH Odyssey 2500. The reagent blank vial should be dark blue.

The 95 % confidence limit of distribution at 1 mg C/l is 1.6 mg C/l and at 5 mg C/l it is 1.1 mg C/l.

4.3.2 Particulate organic carbon (POC)

For the quantification of particulate organic carbon the total organic carbon (TOC) content of the sample is analysed by the HACH method 10129 (HACH, 2001) for samples with a TOC content within the range 0.3-20.0 mg C/l, and the POC content will be calculated by subtracting the DOC content from the TOC content of the same sample.

4.3.3 Total suspended solids (TSS)

The total suspended solids content of the sample is quantified by the HACH method 8006 (HACH, 2001) for samples with a TSS content within the range 0-750 mg/l.

Procedure:

1) 500 ml sample is blended at high speed in a blender for exactly two minutes.
2) The blended sample is poured into a 600-ml beaker.
3) The sample is stirred and 25 ml of it is immediately poured into a sample cell (HACH, 24019-06).
4) The TSS content is quantified using the HACH program 630 Suspended Solids measuring at 810 nm on a HACH Odyssey 2500. Deionised water is used as blank.

4.3.4 Turbidity

The turbidity is measured using a 6035 Turbidimeter (JENWAY) with formazin as standard (Formazin Turbidity Standard 4000 NTU, HACH, 2461-42) or a handheld turbidimeter submersible probe (YSI – 600 OMS) and is reported as Formazin Nephelometric Units (FNU). The lowest measurable turbidity is approximately 0.1 FNU and well within the necessary limit of its use.

4.4 Toxicity testing of treated ballast water

The treated ballast water will be tested for acute, chronic or sub-chronic toxicity using three test organisms representing algae, crustacean (copepods) and fish. The tests with copepods and fish involve long term exposure with frequent renewal. For these tests a batch of the treated ballast water will be stored for the time necessary to complete the tests. Changes of the composition of this sample that may occur during storage is considered as a simulation of changes that will occur after discharge of treated ballast water and, therefore, do not invalidate the tests for this particular purpose. The tests will be conducted for basic and final approval.

4.4.1 Growth inhibition of marine algae

The algae growth inhibition test will be performed according to International Standard ISO 10253: Water Quality – Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*. The diatom *S. costatum* (NIVA-strain BAC 1) will be used as test organism. The ballast water sample will be filtered to remove indigenous algae and spiked with a growth medium concentrate. A dilution series of the sample in pure seawater containing the same nutrient concentrate will be prepared. All batches will be inoculated with *S. costatum* from an exponentially growing laboratory culture and incubated in glass flasks on a shaking table at 20 °C under continuous illumination from fluorescent tubes providing approximately 75 $\mu M\ m^{-2}\ s^{-1}$ of photosynthetic active radiation (PAR). The test will be performed with three replicates at each concentration of ballast water and six control replicates. The cell density will be determined by counting with an electronic particle counter (Coulter Multisizer) after 24, 48 and 72 hours. The growth rate of each culture is calculated and expressed as percentage of the growth rate of control cultures in pure seawater growth medium. If more than 50 % growth inhibition is observed in the ballast water, the EC_{50} and the EC_{10} (i.e. the

concentrations causing 50 and 10 % growth inhibition respectively) will be calculated using non linear regression analysis of the growth rate against log concentration of ballast water.

4.4.2 Acute toxicity of marine copepods

An acute toxicity test will be performed on *Acartia tonsa* according to ISO 14669: Determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*). Copepods that are17-24 old is added to treated ballast water at different dilutions. As control the non treated ballast water is used. The number of survivors is accounted for after 24hours and 48 hours. LC50 and NOEC is estimated based on statistical evaluation of mortality according to the Probit method.

4.4.3 Reproduction of marine copepods

A reproduction test also covering the early life stages of the marine and brackish harpacticoid Copepod *Nitocra spinipes* will be carried out according to Bengtsson and Bergström (1987) and Tarkpea *et al.*(1999). Newly fertilised females will be exposed in ballast water and ballast water diluted in pure seawater for 13 days. A semi-static procedure will be used in which test water is changed every second day. The animals are fed with live algae (*Rhodomonas baltica*). Ten animals are exposed individually at each ballast water concentration and in the seawater control. Survival of the parent females and the number of offspring produced by each female is recorded and compared to the control. If more than 50 % reduction in production of offspring is observed in the ballast water, the EC_{50} and the EC_{10} (i.e. the concentrations causing 50 and 10 % effect, respectively) will be calculated using non linear regression analysis of number of offspring against log concentration of ballast water.

4.4.4 Acute toxicity of fish

The acute toxicity of fish will be performed according OECD guideline 203 modified for marine fish (*Scopthalmus maximus*). As no effects are expected the test will be performed as a Limit test using 20 fish in treated water and 20 fish in control water (non treated ballast water). The test is performed as semi static test with daily renewal of water. Because of the high amount of added organic material the medium will be aired using airstones in order to avoid oxygen depletion. The test will be performed at 16-18 °C.

4.4.5 Juvenile fish growth test

A prolonged exposure test with juvenile turbot (*Scopthalmus maximus*) will be carried out in accordance with the OECD Guideline for Testing of Chemicals 215: Fish Juvenile Growth Test. Juvenile fish in exponential growth phase are placed in test chambers and are exposed to ballast water and ballast water diluted with pure seawater under semi-static conditions. The test duration is 28 days with renewal of the water three times per week. Fish are fed daily. The fish are weighed at the start and end of the test. Effects on growth rates at each treatment will be compared to the control (exposed in pure seawater). If more than 50 % growth inhibition is observed in the ballast water, the EC_{50} and the EC_{10} (i.e. the concentrations causing 50 and 10 % growth inhibition respectively) will be calculated using non linear regression analysis of the growth rate against log concentration of ballast water.

Reference: OECD 203 Modified for turbot juveniles, NIVA method K19.

5. QA/QC checks

5.1 Data Quality Indicators (DQIs)

As part of the statistical analyses and assessment of the quality of data obtained for all performance measurements in the project, five data quality indicators (DQIs) will be used to interpret the degree of acceptability or utility of the data obtained in the project. These are representativeness, accuracy, precision, bias and comparability, and their protocols are described in the following sub sections.

5.1.1 Representativeness

The representativeness will be ensured by doing the following verification procedures:

1. Samples will be withdrawn only from water that has undergone treatment under normal operating conditions.
2. For the performance of the equipment, operating data will be measured at intervals throughout the period of testing ensuring a sufficient quantity of data to detect any change in operation. All alarms and events from the system are continuously logged by the control system. The flow is continuously, online, showed on the screen of the control system. The accumulated flow is calculated and stored in the control system.
3. Complete mixing will be ensured before any withdrawal of samples or in-situ measurement, as verified by the procedure described in section 3.1 point 1).
4. All samples will be taken in triplicates.
5. One control test run will be conducted for each of the test waters to identify any changes in critical parameters not caused by the equipment itself.
6. To estimate the influence of grazers on the level of organisms ≥ 10-50 µm in minimum diameter and to verify the survival of *T. suecica and/or B. submarina* in the control without grazers present, a fraction of the control water will be sieved through a 50 µm mesh to remove the majority of grazing organisms and stored at the same temperature as the rest of the water and in darkness.

5.1.2 Accuracy

Only accredited analytical procedures (according to NS-EN ISO/IEC 17025) will be used to describe critical parameters (e.g. imperative to verify that the required test conditions are fulfilled). The accuracy of these measurements is given in Table 8.

5.1.3 Precision

All samples will be taken in triplicates, and the standard deviation will be calculated. The acceptable analytical precision is ≤ 30 % for critical parameters.

$$\text{Standard deviation, Std} = \sqrt{\frac{(X_i - X)^2}{n-1}} \quad \text{and \% relative standard deviation} = \frac{100 \cdot Std}{X}$$

where X_i = individual analytical result
X = the arithmetic mean of individual analytical results
n = number of measurements

5.1.4 Bias

To minimize possible bias results all samples will be taken in triplicates from the same completely mixed volume of test water. The verification of complete mixing will be done as described in section 3.1 point 1).

5.1.5 Comparability

One control test run will be conducted for each of the test waters as a reference and to identify any changes in critical parameters not caused by the equipment itself.

5.2 Emergency plan

This emergency plan shall be available at all times during testing. All persons in charge of technical equipment or similar connected to the issues listed below will be available at least by telephone during preparation of tests and during testing.

5.2.1 Power failure

The test site is equipped with an emergency power unit that will have start immediately when a power failure incidence takes place. The power unit will have the required capacity to supply lights and pumps of the test unit.

5.2.2 Collapse in organism cultivation

Continuous back-up cultures of the most important organisms will be provided to avoid severe delay from collapse in cultures.

5.2.3 Pumping failure

For pumping of water from the sea to the test-tanks, the test site is equipped with required stand-by pumps if a pump failure should take place. If the main pump of the ballast water treatment system should fail, repair or replacement will be required. Important spare parts will be available at the test site, and agreement with pump dealer regarding repair or replacement will be arranged. The risk of pump failure is very low due to the short pumping intervals during testing (approx. 1 hour).

5.2.4 Identification of *Vibrio cholerae* in test water

In case of the (improbable) identification of *Vibrio cholerae* O1 and O139 in the test water the incident will immediately be reported to the Norwegian Institute for Public Health (NIPH), department for Infectious Surveillance (telephone: 22 04 26 43). Further action on the case will be in agreement with NIPH.

5.2.5 The right to veto between verifier and project group

The testing organization (NIVA) has the main responsible for the test procedure, quality assurance and safety during testing. If a situation should arise were disagreement among the project group members is evident, NIVA by the project manager has the right to decide what measures to be taken. This applies also if a critical situation should emerge.

6. Data reporting, data reduction and data validation

6.1 Approach for data management and evaluation

Data will be recorded in standardized formats and in accordance with the following minimum requirements:

- Data are entered directly, promptly and legibly.
- Data are recorded legibly in ink. All original data records, as appropriate, a description of the data collected, the unit, the unique identification, the name of the person collecting the data and the date and time of data collection. All data will be scanned electronically and filed on a protected computer within 24 hours. All ink recorded data will be stored in a locker with restricted access.
- Any change to the original entry do not obscure the original entry, document the reason for the change and are initialled and dated by the person making the change.

- All deviations from the QAPP will be documented in writing and approved by the person in charge of the quality assurance of that particular part of the project. Documentation and communication include an assessment of the impact the deviation has on data quality.
- Data in electronic format will be included in commercially available programs for word processing, spreadsheet or database processing. Backup of computer databases will be performed on a daily basis.
- All results obtained with the Pureballast treatment system of Alf Laval/Wallenius Water will be handled with confidentiality in accordance with the established "Non-disclosure agreement"

All activities and collected data during testing of the PureBallast treatment system will be logged as summarized in Table 10. For each activity a specially designed log in paper and/or electronic format will be used, shown in Appendix A-G, and will be used for the respective quality assurance evaluation.

Table 10. Log protocols for all activities of the project.

Appendix	Description
A	Total project management
B	Chemical water quality preparation
C	Biological water quality preparation
D	Collection and preservation of samples
E	Logging of *in situ* measurements
F	Evaluation form for organisms >50 µm
G	Evaluation form for organisms ≥10-50 µm
H	Evaluation form for heterotrophic bacteria, coliforms, *E. coli*, Enterococcus group, intestinal *Enterococci*, *Vibrio cholerae* and *Vibrio cholerae* (serotypes O1 and O139).
I	Acute and chronic toxicity of the green algae *Skeletonema costatum*
J	Acute and chronic toxicity of marine copepods
K	Acute and chronic toxicity of juvenile turbot

6.2 Reporting requirements

After the tests have been completed and the data have been evaluated, a report will be prepared and submitted to the Administration. The report will include information regarding test design, methods of analysis and the results of the analysis. Average values and standard deviations will be reported, but all the original data material will be available on request.

6.3 References

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Appendix A – Total project management

Cycle No. (of 5)	Test water

Page 1 of 4

#	Description	Quality assurance	Peformed			Sign.	Verifier	Avvik No.
			Yes	Name	Date			
Preparations – Day 0								
1	Preparation of test tanks			PET				
2	Clarification for the installation of the PureBallast treatment system			Carl				
3	Installation of the PureBallast treatment system			Carl				
4	The PureBallast treatment system clarified for operation			Carl				
5	The chemical water quality requirement fulfilled			CVO				
6	Indigenous organisms harvested			PIJ				
7	Surrogate organisms cultured							
	Artemia salina			ATO				
	B. submarina and/or T. suecica			ATO				
	Heterotrophic bacteria			TGJ				
8	The biological water quality requirement fulfilled			LIL				
Sampling – Day 0								
Day 0 - WST								
9	Homogeneity requirements fulfilled			CVO				
10	Chemical samples collected and preserved							
	DOC, POC, TSS			CVO				
11	Chemical analyses performed							
	Temperature			PET				
	Oxygen			PET				
	Salinity			PET				
12	Biological samples collected							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Microorganisms			TGJ				
Day 0 – TT2								
13	Homogeneity requirements fulfilled			CVO				
14	Chemical samples collected and preserved							
	DOC, POC, TSS			CVO				
15	Chemical analyses performed							
	Temperature			PET				
	Oxygen			PET				
	Salinity			PET				
16	Biological samples collected							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Microorganisms			TGJ				
17	Water for acute toxicity tests collected			ATO				
18	Water for chronic toxicity tests collected			SIX				

#	Description	Quality assurance	Peformed			Sign.	Verifier	Avvik No.
			Yes	Name	Date			
Day 0 – CT2								
19	Homogeneity requirements fulfilled			CVO				
20	Chemical samples collected and preserved							
	DOC, POC, TSS			CVO				
21	Chemical analyses performed							
	Temperature			PET				
	Oxygen			PET				
	Salinity			PET				
22	Biological samples collected							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Microorganisms			TGJ				
Day 0 – CT2*								
23	Samples collected and preserved			LIL				
24	Biological samples collected							
	Organisms ≥10-50 µm			ATO				
Analyses – Day 0								
Day 0 - WST								
25	Chemical samples delivered for analysis			CVO				
26	Chemical analyses performed			CVO				
	DOC							
	POC							
	TSS							
27	Biological analyses performed							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Heterotrophic bacteria			TGJ				
	Coliform bacteria			TGJ				
	Enterococcus group bacteria			TGJ				
	Vibrio cholerae			TGJ				
Day 0 – TT2								
29	Chemical samples delivered for analysis			CVO				
30	Chemical analyses performed			CVO				
	DOC							
	POC							
	TSS							
31	Biological analyses performed							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Heterotrophic bacteria			TGJ				
	Coliform bacteria			TGJ				
	Enterococcus group bacteria			TGJ				
	Vibrio cholerae			TGJ				

#	Description	Quality assurance	Peformed			Sign.	Verifier	Avvik No.
			Yes	Name	Date			
Day 0 – CT2								
32	Chemical samples delivered for analysis			CVO				
33	Chemical analyses performed			CVO				
	DOC							
	POC							
	TSS							
34	Biological analyses performed							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Heterotrophic bacteria			TGJ				
	Coliform bacteria			TGJ				
	Enterococcus group bacteria			TGJ				
	Vibrio cholerae.			TGJ				
Day 0 – CT2*								
35	Quantification of biological treatment performance							
	Organisms ≥10-50 µm			ATO				
Toxicity tests – Day 0								
32	Acute toxicity tests			STK				
	Growth inhibition of *Skeletonema costatum*			ATO				
	Acute toxicity of *Acartia tonsa*			ATO				
	Acute toxicity of *Scoptalamus maximus*			ATO				
33	Chronic toxicity tests			STK				
	Chronic reproduction of *Nitocera spinipes*			SIX				
	Early life stage *Scoptalamus maximus*			SIX				
Securing data – Day 0								
34	All notes scanned and electronically saved			LIL				
Sampling – Day 5								
Day 5 – TT2								
35	Homogeneity requirements fulfilled			CVO				
36	Chemical samples collected and preserved							
	DOC, POC, TSS			CVO				
37	Chemical analyses performed							
	Temperature			PET				
	Oxygen			PET				
	Salinity			PET				
38	Biological samples collected							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Microorganisms			TGJ				
39	Water for acute toxicity tests collected			ATO				
40	Water for chronic toxicity tests collected			SIX				

#	Description	Quality assurance	Peformed			Sign.	Verifier	Avvik No.
			Yes	Name	Date			
Day 5 – CT2								
41	Homogeneity requirements fulfilled			CVO				
42	Chemical samples collected and preserved							
	DOC, POC, TSS			CVO				
43	Chemical analyses performed							
	Temperature			PET				
	Oxygen			PET				
	Salinity			PET				
44	Biological samples collected							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Microorganisms			TGJ				
Day 5 – CT2*								
43	Samples collected and preserved			LIL				
44	Biological samples collected							
	Organisms ≥10-50 µm			ATO				
Analyses – Day 5								
Day 5 – TT2								
45	Chemical samples delivered for analysis			CVO				
46	Chemical analyses performed			CVO				
	DOC							
	POC							
	TSS							
47	Biological analyses performed							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Heterotrophic bacteria			TGJ				
	Coliform bacteria			TGJ				
	Enterococcus group bacteria			TGJ				
	Vibrio sp.			TGJ				
Day 5 – CT2								
48	Chemical samples delivered for analysis			CVO				
49	Chemical analyses performed			CVO				
	DOC							
	POC							
	TSS							
50	Biological analyses performed							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Heterotrophic bacteria			TGJ				
	Coliform bacteria			TGJ				
	Enterococcus group bacteria			TGJ				
	Vibrio sp.			TGJ				
Day 5 – CT2*								
51	Quantification of biological treatment performance							
	Organisms ≥10-50 µm			ATO				

Appendix B – Chemical water quality preparation

Page 1 of 1

Purpose: Preparation of chemical water quality of test water		Water type	
Name:		Seawater:	
Date:		Brackish water:	

Before any additions		
In situ measurements		
Temperature	°C	
DO	mg O/l	
Salinity	PSU	

Additions								
Compound	added amount	Estimated additional concentration			Estimated final concentration			
		TSS	DOC	POC	TSS	DOC	POC	
Kaolin								
Cryst. cellulose								
Lignin								

Test of homogeneity									
		Unit	Cal.	Sampling point in water column				Average	Std
Tank	Parameter			center/perif.	upper	mid	lower		
WST	Turbidity	NTU		center					
				periphery					
TT2	Turbidity	NTU		center					
				periphery					
CT2	Turbidity	NTU		center					
				periphery					

Turbidity instrument:	Calibrated:

Rapid measurements after all additions			
TSS		Instrument:	
Sample No.	Sampling point	Concentration (mg/l)	Average
I			
II			
III			

	Date, signature:
Operator	
Verifier	

Appendix C – Biological water quality preparation

Purpose: Preparation of biological water quality of test water		Water type	
Name:		Seawater:	
Date:		Brackish water:	
Sample id.:			

Harvested indigenous organisms		***Volume added:***	
	Observed species	Phylum	
Organisms >50 μm			
Organisms ≥10-50 μm			

Cultured surrogate organisms			
	Concentrate		
	Measured conc.	Volume added	Expected final conc.
Artemia salina			
B. submarina and/or T. suecica			
Heterotrophic bacteria			

	Date, signature:
Operator	
Verifier	

Appendix D – Collection and preservation of samples

Page 1 of 2

Sample Id.	Sample point
WST	Prepared test water
TT2	Test tank 2
CT2	Control tank 2

Purpose: Collection and preservation of samples				Water type		
Name:				Seawater:		
Date:				Brackish water:		
Sample id.:						
	P1	P2	P3	Volume	Filtration/ Preservation	Sign.
Chemical samples						
Day 0 – WST						
DOC						
POC						
TSS						
Day 0 – TT2						
DOC						
POC						
TSS						
Day 0 – CT2						
DOC						
POC						
TSS						
Biological samples						
Day 0 – WST						
Organisms > 50 µm						
Organisms ≥10-50 µm						
Bacteria						
Day 0 – TT2						
Organisms > 50 µm						
Organisms ≥10-50 µm						
Bacteria						
Day 0 – CT2						
Organisms > 50 µm						
Organisms ≥10-50 µm						
Bacteria						
Toxicity samples						
Growth inhibition of green algae						
Reproduction of marine copepods						
Juvenile fish growth test						

	Date, signature:
Operator	
Verifier	

Sample Id.	Sample point
WST	Prepared test water
TT2	Test tank 2
CT2	Control tank 2

Purpose: Collection and preservation of samples					Water type		
Name:					Seawater:		
Date:					Brackish water:		
Sample id.:							
	P1	P2	P3	Volume	Filtration/ Preservation	Sign.	
Chemical samples							
Day 5 – TT2							
DOC							
POC							
TSS							
Day 5 – CT2							
DOC							
POC							
TSS							
Biological samples							
Day 5 – TT2							
Organisms > 50 µm							
Organisms ≥10-50 µm							
Bacteria							
Day 5 – CT2							
Organisms > 50 µm							
Organisms ≥10-50 µm							
Bacteria							
Toxicity samples							
Growth inhibition of green algae							
Reproduction of marine copepods							
Juvenile fish growth test							

	Date, signature:
Operator	
Verifier	

Appendix E – Logging of in situ measurements

Page 1 of 1

Sample Id.	Sample point
WST	Prepared test water
TT1, TT2	Test tank 1, 2
CT1, CT2	Control tank 1, 2

Purpose: Logging of *in situ* measurements			Water type	
Name:			Seawater:	
Date:			Brackish water:	
Sample id.:				

Time of measurement	**Temperature** °C	**pH** -	**Dissolved oxygen** mg O/l	**Salinity** PSU

	Date, signature:
Operator	
Verifier	

Appendix F – Evaluation form for organisms >50 µm

Page 1 of 1

Purpose: Preparation of biological water quality of test water				Water type	
Name:				Seawater:	
Date:				Brackish water:	
Sample id.:					
	Observed species			**Phylum**	
	Live	**Dead**			

	Date, signature:
Operator	
Verifier	

Appendix G – Evaluation form for organisms ≥10-50 µm

Page 1 of 1

Purpose: Preparation of biological water quality of test water		Water type	
Name:		Seawater:	
Date:		Brackish water:	
Sample id.:			

	Observed species			**Phylum**
	Live	**Dead**		

	Date, signature:
Operator	
Verifier	

Microscopy of CFDA incubated samples					
Sample id:		Analysed by, sign	Date:		
		A. Tobiesen			
Organisms	sub. Sample 5 ml	sub. Sample 5 ml	sub. Sample 5 ml	sub. Sample 5 ml	sub. Sample 5 ml
sub. Sample 5 ml	sub. Sample 5 ml	sub. Sample 5 ml	sub. Sample 5 ml	Rest volume	Total

NORSK INSTITUTT FOR VANNFORSKNING

Bakteriologiske analyseresultater

J 2 = NS 4788 (Kolt-37°) | J 4 = NS 4792 (Kolt-44°) | J3 =NS 4791 (Klm-20) | J6 = NS 4793 (Strept)

Oppdragsnr.: Lab.kode:	Prøvetak. dato: Prøvetak. tid:	Lagringstemp	Mottatt NIVA dato: Analysert dato:	Rekvisisjonsnr:

METODE :											
Prøve nr.									Sign	Inkubator:	
Fortynningsgrad										Middeltemp	
Testporsjon, mL (V)										Temp. min.	
Fortynningsfaktor, (F)										Temp. max.	
Telte kolonier, antall (C)										Tid inn:	
Middelverdier										Tid ut:	
Antall pr. st. vol. (Vs)											
95 % konfidensintervall											
METODE NR:										Inkubator:	
Fortynningsgrad										Middeltemp	
Testporsjon, mL (V)										Temp. min.	
Fortynningsfaktor, (F)										Temp. max.	
Telte kolonier, antall (C)										Tid inn:	
Middelverdier										Tid ut:	
Antall pr. st. vol. (Vs)											
95 % konfidensintervall											
METODE NR:										Inkubator:	
Fortynningsgrad										Middeltemp	
Testporsjon, mL (V)										Temp. min.	
Fortynningsfaktor, (F)										Temp. max.	
Telte kolonier, antall (C)										Tid inn:	
Middelverdier										Tid ut:	
Antall pr. st. vol. (Vs)											
95 % konfidensintervall											

Forberedelse:	
Utførelse:	

g:\felles\bakt\baktarkx

Appendix I – Acute toxicity of the green algae Skeletonema costatum (page 1 of 1)

Algetest oppsettingsskjema til metodene K4, K5 og K6

Testmetode: **Lab. kode:**

Startdato:

Testalge: **Klon:**

Podekultur

Start dato	mill/l	vokst til	v.hast.	podevol.	til volum	mill/l nominelt		mill/l tellt	
Sign:		Dato:				Sign:			

Fortynningsmedium (Sjøvannsbatch ved marine tester)

Stamløsninger av teststoff - ev. forbehandling

nr.	kons.	veid/målt	av	vekt/pip.	fort. til	dato	anmerkning	sign.
I								
II								
III								
IV								

Testløsninger

Kons.	veid/målt	stamløsn.	vekt/pip.	Podet ml	pipette	fortyn. til	pH start	pH slutt
Kontroll								
								Dato
Inkubering startet:				Dato:		Sign.:		sign.

Lys opp:		Lys ned:		Instrument:	Sensor:
Lysmåling dato:		Sign.:			

Temp. max:		min:		Dato:	Sign.

	Date, signature:
Operator	
Verifier	

Appendix J – Acute toxicity of marine copepods (page 1 of 1)

Akutt-test med Acartia sp., oppsettingsskjema til metode K13

Testmetode: K13 **Labkode:**

Startdato: **Dato for filtrering av testdyr:**

Fortynningsmedium

GF/C-filtrert sjøvann justert til 32 promille salinitet	Sjøvannsbatch:	

Stamløsninger av teststoff - ev. forbehandling

nr.	kons.	veid/målt	av	vekt/pip.	fort. til	dato	anmerkning	sign.
I								
II								
III								
IV								

Testløsninger

Kons.		veid/målt		stamløsn.	vekt/pip.	fort. til	pH start	anmerkning

Oppsatt av: **Sign.** **Temp. min.**

Temp. max

Appendix K – Acute toxicity of juvenile turbot; setup form for method K19

Page 1 of 3

Test method: K19 Lab code:

Starting date: Fish from:

Climatic room: Number of fish per aquarium:

Dilution medium:

Stamløsninger av teststoff eventuallt forbehandling

Nr.	Kons.	Veid/målt	av	Vekt/pip.	Fort. til	dato	Anmerkning	sign
I								
II								
III								
IV								

Testløsninger

nr.	Kons.	Veid/målt	Stamløsn.	Fort. til	pH start	pH slutt	O_2 slutt	sign/dato

Termometer nr:

Oppsatt av: Sign. Temp. min

Testansvarlig: Sign. Temp. max

Appendix K – Acute toxicity of juvenile turbot; test protocol for method K19

DATA FORM: PARCOM\TURBOT.JUV\MOTRAL.DAT

TEST PROTOCOL: OECD 203 Modified for turbot juveniles

		Sign
Test performed by:		
Responsible for test:		

MORTALITY DATA

Test Substance:

Lab. Code

TEST DATE:

Concentration of Test substance mg/l	No. fish per tank	No. of dead fish				Notes on abnormal behaviour
		24 h	48 h	72 h	96 h	
CONTROL						
Date:						Date:
Signature:						Sign:

Weight of dead fish											middl.
Length of dead fish											middl.

Appendix K – Acute toxicity of juvenile turbot

Study director:		
Test description:	K19,	Fish acute toxicity test (OECD 203): 96 h LC50
Test Organism:		Juvenile turbot, Scophtalamus maximus
Origin of test organism:		
Date of arrival:		
Age on arrival:		
Age when used in test:		
Holding conditions:	pH	
	Temp	
	Salinity	
	Dissolved O2	
	Flow rates	
	Mortality last 7 days	
Feeding regime:		
Test containers (size, material):		
No. of fish per container:		
Preparation of test substance:		
Concentrations tested:		
Comments:		

	Date, signature:
Operator	
Verifier	

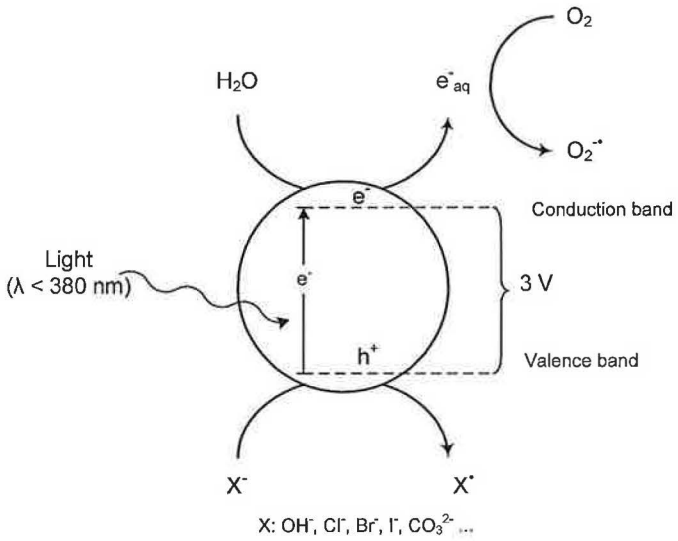
Appendix L – Process Description

This appendix describes the chemical features and discharge characteristics of the Wallenius-AOT process.

Chemical features

In sea-water, containing high concentrations of halide anions, charge-transfer absorptions will block all photons < 220 nm. (Platzman and Frank 1954, Treinin and Hayon 1975). This prevents any formation of ozone and related problems

Several metal-oxides exhibiting semi-conducting properties can be used as photo-catalysts in AOT-applications. Many of these applications are based on the specific properties of the anatase phase of titanium dioxide, TiO_2. In this material the absorption of a UV-photon of wavelengths below 387 nm can create a hole-electron pair of significant life-time (Kamat 1993, Legrini *et al.* 1993, Tada 2002, Fujishima *et al.* 2000, Diebold 2003).



If the hole $\oplus$ and the electron $\ominus$ can reach the surface of the photo-catalyst, they may cause oxidation (hole) and reduction (electron) transformations in the surrounding medium. In a fundamental study it has been shown that the electron enters the water medium becoming a solvated electron (Rothenberger *et al.* 1985), a state of well known chemistry with reaction times on the pico- to micro-second time scales (Baxendale and Busi 1982).

Typically, the solvated electron is trapped by oxygen in a diffusion controlled reaction forming superoxide, a free radical of low reactivity.

$$e_{aq}^- + O_2 \rightarrow O_2^{\bullet -}$$

Eventually, superoxide may disproportionate by formation of hydrogen peroxide:

$$2\,O_2^{\bullet -} + 2\,H^+ \longrightarrow H_2O_2 + O_2$$

Under the conditions prevailing in the AOT-treatment unit, hydrogen peroxide may undergo homolytic or reductive cleavage leading to formation of hydroxyl radicals, $^{\bullet}OH$:

$$H_2O_2 \rightsquigarrow 2\,{}^{\bullet}OH$$

$$e^-_{aq} + H_2O_2 \rightarrow HO^\bullet + OH^-$$

These reactions can be boosted by addition of hydrogen peroxide to the water entering the AOT-unit.

Hydroxyl radicals have superior oxidizing power and react exceedingly fast with most organic and inorganic constituents of sea water, *e.g.* with chloride ions (Baxendale and Busi 1982, Buxton *et al.* 1988, NDRL/NIST Solution Kinetics Database 2005).

When the positive hole, h+, reaches the surface it can oxidize adsorbed species on the particle surface. Thus, this reactivity is dependent on the adsorption of ions and other matter to the surface, goverened by the surface z-potential, which is pH-controlled (Tada 2002).

The hole, h^+, is a very strong one-electron oxidant that can react directly with adsorbed organic pollutants (including micro-organisms) or generate different oxidising radical species depending on the composition of adsorbed chemical species.

$$\oplus + X^- \longrightarrow X^\bullet$$

A typical sea-water contains about 0.5 M NaCl, 0.84 mM Br^- and 2.33 mM HCO_3^- (Horne 1969). Due to the high chloride concentration it is likely that the main adsorbed species on the photocatalyst surface is the chloride ion. Thus, a hole reaching the photo-catalyst surface probanly oxidizes a chloride ion to a chlorine atom:

$$\oplus + Cl^- \longrightarrow Cl\cdot$$

This initial step is followed by a series of subsequent redox-reactions (Xiao-Ying 2004):

$$Cl\cdot + Cl^- \rightleftharpoons Cl_2^{\cdot-}$$

$$Cl\cdot + H_2O \rightleftharpoons H^+ + ClOH^{\cdot-} \rightleftharpoons Cl^- + \cdot OH$$

Under prevailing conditions, i.e. near neutral pH and high concentration of chloride ions, predominant radicals acting in the system are dichloridyl ($Cl_2^{\cdot-}$), chlorbromidyl ($ClBr^{\cdot-}$), dibromidyl ($Br_2^{\cdot-}$) and carbonate ($CO_3^{\cdot-}$) radicals.

$$Cl_2^{\cdot-} + 2\ Br^- \rightarrow Br_2^{\cdot-} + 2\ Cl^-$$
$$Cl_2^{\cdot-} + CO_3^{2-} \rightarrow CO_3^{\cdot-} + 2\ Cl^-$$

These radicals are strong one-electron oxidants. Paradoxically, they are probably more bio-active than hydroxyl radicals because they may selectively attack essential proteins in the cell membranes of micro-organisms, a reaction which may lead to lysis and subsequent death (Engel *et al.* 1974, Ivanovskii and Mitrofanov 1978, Nogueira *et al.* 1998, Rijstenbil 2003, Zaafrane *et al.* 2004, Rijstenbil 2005).

UV-photons (□ about 260 nm) have also a direct lethal effect by destroying DNA., *e.g.* by pyrimidine lesions. Thus, free radicals and UV-photons kill micro-organisms synergistically. In addition, persistent pollutants, *e.g.* NDMA (N-nitrosodimethylamine), ATZ (atrazine) are oxidized and organo-halogen compounds like DDT and PCB can be reductively dechlorinated by trapping electrons on the photo-catalyst surface (Ahmed *et al.* 1999):

$$\ominus + R\text{-}Cl \longrightarrow R^\cdot + Cl^-$$

A problem related to ozone treatment of sea water is a significant formation of bromate, a suspected carcinogen. This reaction may proceed by way of an oxygen atom transfer mechanism. In the photo-catalytic process formation of bromate, BrO_3^-, is unlikely to occur, since this would require multiple one-electron oxidation steps. Moreover, the utilization of photocatalysts is one of the attractive methods for the removal of BrO_3^-. The reduction of BrO_3^- on a TiO_2 photocatalyst under UV irradiation is a well-known reaction, and bromate has even been used as an additive to improve the oxidation efficiency of TiO_2 photocatalysts (Oosawa and Gretzel 1988, Al-Ekabi *et al.* 1993, Mills *et al.* 1996, Lindner *et al.* 1997, Noguchi *et al.* 2003)

It should be pointed out that the radicals produced in the AOT-treatment unit are not different from those naturally formed by sun light in the surface layer of see-water. In this case, photo-cleavage of hydrogen peroxide and/or photo-oxidation of nitrate/nitrite ions may be the source of radical formation.

Discharge characteristics
The basic process operates without addition of chemicals using the synergistic effects of in situ produced free radicals and UV-photons to inactivate micro-organisms. In this system, the active substance is the anatase phase of TiO_2, which under UV-irradiation is classified as a biocide by the biocide directive of the European Union. *Per se*, TiO_2 is non-toxic as shown by its use in re-constructive surgery to obtain bone-compatible links for Ti-implants such as teeth and hip-joints (Pan *et al.* 1996, Diebold 2002). TiO_2 is also a widely used component in paints and cosmetic products. The action of reactive free radicals is of temporal nature, *i.e.* these species are so short-lived (micro-milli second time-scale) that they are not observable outside the AOT-treatment unit (Baxendale and Busi 1982, Buxton *et al.* 1988, NDRL/NIST Solution Kinetics Database 2005).

If required, the effects can be further amplified by addition of minor amounts of hydrogen peroxide before the AOT-treatment. More information to be added.

Even though the process is not based on the addition of toxic substances, it can not be excluded that treated ballast water may contain some bio-active reaction products. Therefore, treated ballast water must be tested for toxicity, specifically for:

- Chloro-organic compounds
- Bromo-organic compounds
- A general toxicity analysis (oxidized organics)

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Appendix M – Description and the PureBallast system and its components

The PureBallast product is a complete system, composing of three main functions:

- Filter (50 µm)
  For removal of larger particles and organisms as well as to reduce the level of sediments in the ballast water tanks.
- Wallenius-AOT (Advanced Oxidation Technology) Unit
  A patented system product using naturally occurring photo-processes in the surface layer of sea-water. The Wallenius-AOT process acts on two levels: *in-situ* formation of reactive free radicals, and direct photo-effects. These two levels synergistically inactivate micro-organisms.
- Control and Auxiliary Equipment including sampling points
  The control system and transformer control the system and supply all items with power. The auxiliary equipment controls the flow and measure different alarm levels. The system also composes a CIP unit which is an automatic service device that cleans the quartz sleeves covering the UV-lamps after each Ballast or Deballast sequence. This operation is done to avoid scaling from seawater contaminants which would otherwise reduce the efficiency of the Wallenius-AOT Module.

Filter

S201-650 µm automatic back flushing filter

Wallenius-AOT unit

V201-19.1 Inlet valve for the Ballast water into the Wallenius-AOT Module. (DN 100)

V201-20.1 Outlet valve for the Ballast water from the Wallenius-AOT Module. (DN 100)

RV201-23.1 Relief valve to avoid if too high pressure in the Wallenius-AOT Unit would occur. The relief valve is set to 7 bars.

V321-2.1 The valve used for soft start of the Wallenius-AOT and circulation of CIP fluid to avoid pressure peaks in the Wallenius-AOT unit. (DN 40)

V321-3 Air relief valve for efficient drainage of the Wallenius-AOT Module.

V320-4.1 and V320-5.1 The valves should be open during the CIP process and during drainage of the Wallenius-AOT unit. The CIP process is circulating the CIP fluid from bottom to the top of the Wallenius-AOT module and then re-circulated to the CIP tank.

LS201-29 Level switch that detects the flow to get a signal when the AOT unit is full during soft start.

Control and Auxiliary Equipment including sampling points

Control System An overall control system that controls the process, log all data and give alarms.

Transformer	Transformer that supplies all units with power.

Auxiliary Equipment

V201-3	Valve opened at ballasting for water flow through filter. Closed at deballasting for by-passing of the filter. Also used to open or close the total PureBallast system. (DN 250)
V201-9	Valve opened at ballasting for by-passing of the filter. Closed at deballasting. Also used to open or close the total PureBallast system. (DN 250)
V201-32	Outlet valve of treated ballast water. Opened both during ballasting and deballasting.
V201-8	Regulating valve to create a pressure difference over the filter to ensure efficient back-flushing, 2 bars needed. (DN 200)
IP201-7	Regulator for adjusting the valve V201-8.
PT201-27	Pressure Transmitter for controlling the pressure difference over the filter to have it as stable as possible around 2 bars.
V201-26	Valve to make it easier to exchange pressure transmitter (PT201-27).
FIT201-1	Flow meter that measures the amount of ballasted and deballasted water. This data is logged in the control system and adjusts the air regulation in the process.
PT201-16	Pressure Transmitter for adjusting the air regulation in the process.
V201-15	Valve to make it easier to exchange the pressure transmitter (PT201-16).
PI201-18	Pressure Gauge to be able to see the pressure direct in the process.
PD201-40	Protection for the Pressure Gauge to increase its life time. (Not standard in the PureBallast system)
V201-17	Valve to make facilitate exchange of the Pressure Gauge (PI201-18).

Sampling points

Q201.1	Sampling point for inlet water
Q201.2	Sampling point for outlet water
Q201.3	Sampling point for water after the filter and before Wallenius-AOT (not included in commercial system)
V201-2	Valve for sampling point Q201.1 (DN 50)
V201-43	Valve for sampling point Q201.3 (DN 50) (not included in commercial system)
V201-44	Valve for sampling point Q201.2 (DN 50)
V201-25	Injection and sampling point (Not standard in the PureBallast system)
V201-37	Injection and sampling point (Not standard in the PureBallast system)

CIP (Cleaning In Place)

T320-10	Tank for storage of CIP liquid between cleaning cycles and circling of CIP fluid.

P320-1 Pump to circulate the CIP fluid.

LS320-7 Mini Squing to detect high flow in order to detect leakage of Ballast water into the CIP. (High Level Alarm)

LS320-8 Ultra sound detector to measure the level in the CIP tank. (Low Level Alarm) This is a safety component to protect the pump from overload and to detect potential leakages, correct opened and closed valves, that the tank has been filled with liquid etc. (Low Level Alarm)

V460-3 Manual valve for drainage of the CIP tank.

V320-2 Valve opened during the CIP process, draining of CIP

V321-1 Valve opened during the CIP process.

V460-2 Valve for drainage of Ballast water

FLC320-11 Hose for CIP liquid from CIP tank to Wallenius-AOT.

FLC321-4 Hose for CIP liquid from Wallenius-AOT to CIP tank.

FLC460-4 Hose for drainage of the CIP tank.

FLC460-5 Hose for drainage of the Wallenius-AOT

Hoses are used to isolate the CIP tank from vibrations and to make the installation more flexible since the hoses easily can be extended or shortened.